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ORIGINAL ARTICLE

Survey of *Salmonella* infections in broiler farms in Iran during 2013-2014: a cross-sectional study

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ABSTRACT

Background and Objectives: The aim of this study was to determine the prevalence of *Salmonella* at broiler breeder farms of Iran and investigate the factors underlying salmonellosis in these farms. This is a cross-sectional investigation conducted in 23 provinces of Iran.
Materials and Methods: Fecal samples were collected from 139 broiler breeder farms in the country and standard bacteriological tests were carried out on the samples for the isolation of *Salmonella*. The serological tests were then applied for the samples that were positive in the bacteriological test. The information on the sampled farms extracted from the Iran GIS-VET Monitoring and Surveillance System was used for the analysis of the risk factors.
Results: A total of 11 farms out of the 139 sampled farms were infected with *Salmonella* with the largest number of infected cases related to Tehran and Fars Provinces.
Conclusion: The statistical analysis results showed that flocks with older ages and farms with larger number of houses are at greater risk of *Salmonella* infection.

Keywords: *Salmonella*; Salmonellosis; Broiler breeder; Poultry; Risk factors

INTRODUCTION

Salmonella affects a wide range of animal hosts. Human salmonellosis is one of the important zoonotic disease. Enteric fever, typhoid fever, food infection, diarrhea and gastroenteritis are various forms of Salmonellosis in human. The global burden of infection with non-typhoid *Salmonella* is annually estimated to be 93.8 million cases of gastroenteritis and 155,000 deaths (1).

Salmonella infection in poultry are considered to be of great public health importance. Several studies performed in the USA and Europe demonstrated that consumption of undercooked chicken and eggs

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infected with *Salmonella* is one of the major sources of Salmonellosis in humans (2); however, the origin and the modes of transmission of this infection are not well studied in developing countrie (1).

The bacterium *Salmonella* is able to transfer vertically. It can consequently leads to the contamination of hatcheries and broiler houses. The infection may persist throught the supply chain and during slaughter and meat packing and cause a major risk for the consumers.

Some stereotypes of *Salmonella*, including *Salmonella* Enteritidis, may be colonized in the oviducts and infect them during the formation of eggs, but all *Salmonella* serotypes have the potential to penetrate the eggs through the shell after their formation and infect the produced eggs (3).

Salmonella can be transmitted to eggs vertically and from the brood. In addition, poultry farms may be infected in different ways, including, infected food and water, and entry of rodents and insects carrying *Salmonella*. Infected birds also transmit the infection to their surrounding environment and birds (2, 4). It is shown that although there are different ways of infection of broiler chicken farms, the major way of their infection is the infection of hatchery by the *Salmonella* bacteria (5).

Any controlling measure taken against *Salmonella* needs to be associated with the top-down policy; i.e. infection should be controlled first at the level of breeders, so that infection cannot be transmitted to lower levels of breeding. *Salmonella* control requires formulating a surveillance program for regular sampling and taking appropriate control measures. In this regard, the first step to formulate a surveillance program is to determine the infection status and the factors underlying infection (6, 7).

The aim of this study was to determine the prevalence of motile *Salmonella* at broiler breeder farms of Iran and to investigate the factors underlying Salmonellosis in these farms. This study was conducted throughout the country and the information obtained in this study can be helpful for the policy-makers of poultry health and industries associated with the production of broiler breeders and chickens.

MATERIALS AND METHODS

Sampling. This cross-sectional study was conducted from June 2013 to March 2014 in 23 provinces of the country. It is noteworthy that 25 provinces of Iran have broiler breeder farms, and all of these 25 provinces were participated in this project except for Khuzestan and Ardabil Provinces. A total of 139 broiler breeder farms were selected as the research sample using the stratified random sampling method and based on the number of farms in each province. Two fecal samples, 150-gram each, were collected from every breeding poultry house and then transferred to the central laboratory of the province within 24 hours. Attempts were made to collect the fecal samples from different parts of the poultry houses so that these two samples of feces could represent the feces of all parts of the poultry house.

Isolation and identification of *Salmonella*. Each fecal sample (25 g) was added to Buffered Peptone Water (225 ml, 25°C) and incubated at 37°C for 18 h. Then 1 ml of each diluted sample was transferred to 9 ml of selenite cystine broth and Rappaport-Vassiliadis broth, and were incubated (42°C/24 h for Rappaport-Vassiliadis medium and 37°C/24 h for Selenite-Sistein medium). At the next stage (Isolation), Xylose Lysine Desoxycholate (XLD) agar, McConkey agar, and *Salmonella-Shigella* (SS) agar as Differential-Selective media were used to detect probable *Salmonella* colonies after incubation the plates at 37°C for 24-48 h. Suspected colonies (colorless colonies in McConkey and SS agars; pink colonies with black centers in XLD agar) were picked fo identification. The suspected colonies were cultured in Triple Sugar Iran (TSI) agar, Lysine Iron (LI) agar, Urea agar, and tested by standard methods including IMVIC and Oxidase tests. For final confirmation, positive microorganisms in the former chemical tests underwent serological tests. Somatic (O) polyvalent antiserum was used for serogrouping. Each *Salmonella* isolated colonies of the pure culture of TSI slants were mixed with 8.5% saline onto a slide. After checking for autoagglutination, one drop of O polyvalent antiserum (A-S) was added and mixed with the bacterial emulsion and the result was read on the black background, in front of the lamp. The agglutination that occurred before 2 minutes, was considered positive. In this case, the experiment was repeated for all the contained antisera of the polyvalent antiserum to identify the isolated *Salmonella* serogroup (8-12).

Risk factors. In this study, eight possible risk factors associated with the occurrence of *Salmonella*

infection was assessed. The season of sampling was documented as well as information about breed and age of the brood at the time of sampling. We also obtained data about farm managements (including single or multi age systems, manure collection techniques and presence or absence of appropriate farm fencing) and size of the farm (the capacity of the farm and the number of poultry houses).

Information related to these factors on the sampled chicken farms was adapted from the Iran GIS-VET Monitoring and Surveillance System (a nationwide information database for poultry farms which is only available for Iranian Veterinary organization) and was used for the analysis of the risk factors. Farms with at least one bacteriological positive samples were considered infected.

Statistical analysis. This information and the results of the *Salmonella* detection tests were entered into the SPSS ver. 21 for statistical analysis. The relationship between Salmonellosis and the qualitative variables under study was tested using the Chi-square test. The odds ratio (OR) and its confidence interval were calculated and reported using univariate logistic regression. The variables significantly correlated with Salmonellosis (P <0.1) were selected for the final model at this step. At the next step, the correlation between the independent variables was examined, and one of the correlated variables was selected to be entered into the final model considering its biological fitness. The final multivariate regression model was obtained using the forward selection.

RESULTS

A total of 11 farms (7.9%) out of the 139 breeder farms studied in 2013-2014 were positive for Salmonellosis with 95% confidence intervals (3.0% to 12.4%). The positive samples belonged to Tehran, Fars, Mazandaran, Kerman, West Azerbaijan and Chaharmahal and Bakhtiari Provinces with the large number of positive cases in Tehran and Fars compared to the other provinces (Fig. 1).

The average age of the birds at the farms where they were sampled was 46.90 with the standard deviation of 20.51 weeks. Among the 11 positive samples, five samples belonged to the serogroup D (45.4%), three samples to serogroup C (27.3%), and two samples to serogroup B (18.2%), and the serogroup of one of the

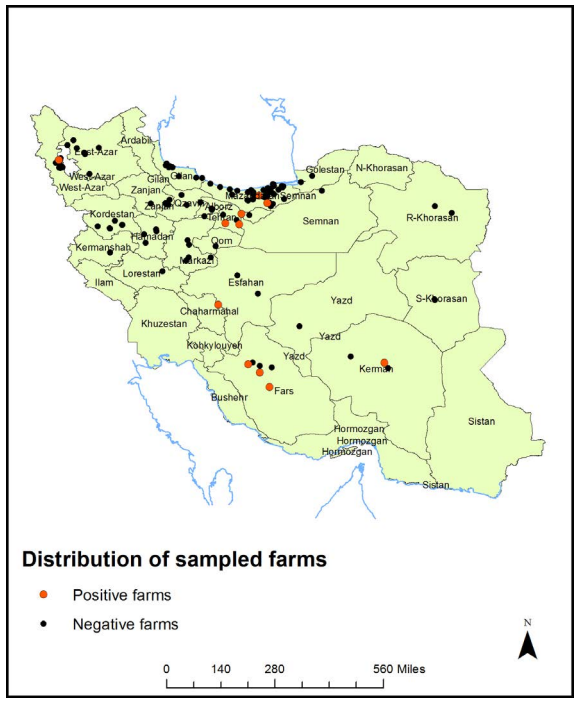

Fig. 1. Distribution of *Salmonella* positive and *Salmonella* negative sampled farms.

samples (9.1%) was unknown. The results indicated that the infected and the uninfected chicken farms had no significant statistical difference in terms of their Farm capacity, breed, manure collection techniques, presence or absence of appropriate farm fencing and season of sampling (P>0.05) (Tables 1 and 2). The final regression model showed that the infected farms were significantly different from the uninfected ones in the number of their poultry houses (P=0.037) and age (P=0.019) (Table 3).

DISCUSSION

It is extremely important to detect infection with *Salmonella* at broiler and breeder farms as it leads to infection in broiler chickens and then into the produced poultry meat. This study revealed that 7.9 percent of the breeder farms under study were infected with *Salmonella* sp. A study conducted in 2011 in Iran estimated the prevalence of infection with *Salmonella* at broiler breeder farms as 9.7 percent (13). Various studies conducted over the last ten years show the prevalence of infection with *Salmonella* at broiler chicken farms in Iran is between 22.5 and 64.2 percent (14, 15), and the prevalence of infection

Table 1. Distribution (Frequancy (Percentage)) and mono-variable analysis of qualitative explanatory variables of *Salmonella* sp. contamination in broiler breeder farms (139 farms).

Definition of variables	*Salmonella* sp. negative	*Salmonella* sp. positive	Odds Ratio	95% Confidence	P Value
	n (%)	n (%)	(OR)	interval (CI)	
Multiple age system[c]					
Yes	116 (95.1)	8 (72.7)	7.2	1.8-28.8	0.005*
No	6 (4.9)	3 (27.2)			
Appropriate farm fencing[c]					
Yes	119 (97.5)	11 (100.0)	0	0-15.2	0.599
No	3 (2.4)	0 (0.0)			
Farm has damping equipment[c]					
Yes	115 (94.3)	11 (100.0)	0	0-6.2	0.414
No	7 (5.7)	0 (0.0)			
Season of sampling[a]					
Spring	22 (17.2)	1 (9.1)	-	-	-
Summer	45 (35.1)	6 (54.5)	2.9	0.3-140.9	0.313
Autumn	36 (28.1)	3 (27.3)	1.8	0.1-100.5	0.605
Winter	25 (19.5)	1 (9.1)	0.9	0.1-72.2	0.929
Breed[bc]					
Ross	98 (80.3)	7 (63.6)	-	-	-
Cobb	18 (14.7)	4 (36.4)	3.1	0.6-13.7	0.080*
Hy-Line	2 (1.6)	0 (0.0)	0	0-30.7	0.709
Arbor Acres	2 (1.6)	0 (0.0)	0	0-30.7	00.709
Arian	1 (0.8)	0 (0.0)	0	-	0.789
Bonz	1 (0.8)	0 (0.0)	0	-	0.789

The odds ratio (OR) and its confidence interval were calculated and reported using univariate logistic regression.
*Significant P values with significance level of 0.100.
[a]Spring regarded as baseline category.
[b]Ross regarded as baseline category-Arian and Bonz was not included in the analysis because of low sample size.
[c]For these variables data was only available for 133 farms.

Table 2. Distribution (Mean ± SD) and mono-variable analysis of quantitative explanatory variables of *Salmonella* sp. contamination in broiler breeder farms (139 farms).

Definition of variables	*Salmonella* sp. negative	*Salmonella* sp. positive	Odds Ratio (OR)	95% Confidence interval (CI)	P Value
Age (Weeks)	45.3 ± 19.9	65.0 ± 19.3	1.0	1.0-1.0	0.005*
Poultry- size (no. of broiler breeders of farm)	35564.4 ± 17000.0	42727.3 ± 20386.7	1.0	1.0-1.0	0.195
Number of poultry houses of the farm	5.9 ± 3.4	9.0 ± 5.2	1.2	1.0-1.36	0.014*

The odds ratio (OR) and its confidence interval were calculated and reported using univariate logistic regression.
*Significant P values with significance level of 0.100.

with *Salmonella* in the chicken slaughterhouses were shown to be between 11.6 and 47 percent (16, 17). We think that our study probably underestimate the true prevalace of *Salmonella* infection. It was the first national attempt to determine the prevalence of *Salmonella* infection in broiler breeder farms all over the country, so we encountered several problems to coordinate sampling and laboratory techniques in different provinces. These pitfalls might cause failing to detect *Salmonella* in some of the infected farms.

Table 3. Final mixed logistic-regression model of risk factors for *Salmonella* sp. contamination of in broiler breeder farms (139 farms).

Definition of variables	Odds Ratio (OR)	95% Confidence interval (CI)	P Value
Multiple age system age system	4.9	0.8-30.9	0.87
Cobb (Breed)	0.4	0.4-3.8	0.440
Age (Weeks)	1.0	1.0-1.0	0.019*
Number of poultry houses of the farm	1.2	1.0-1.4	0.037*

Results of multivariable logistic regression analysis -Intercept = 0.006
*Significant P values with significance level of 0.05.

Salmonellosis can also increase along the production chain and ultimately leads to the contamination of the chicken meat used by consumers (18).

In this study, Serogroups D (45.4%) and C (27.3%) had the highest rate of infection, respectively, but the serotypes were unfortunately not determined due to the unavailability of appropriate diagnostic facilities and reagents. In another similar study performed in 2011 in Iran, the highest rate of infection in the poultry breeders belonged to Serogroup D (13). *Salmonella* Enteritidis, the major *Salmonella* serotype causing diseases in humans, belongs to Serogroup D. *Salmonella* Infantis and *Salmonella* Newport are two important serotypes shown in North America and Europe to be among the major causes of Salmonellosis in humans which are in Serogroup C (19). Accordingly, infection serogroups D and C can be important in terms of public health. One limitation of this study was that the PCR test was not performed on *Salmonella* isolates. This test could considerably help to detect the important serotypes causing this infection.

The present study showed that the age of brood was related to Salmonellosis at broiler breeder farms in Iran, however; this association was very slight. It is in accordance with previous obseravtions which shows the older the chickens, the more likely it will be for them to be infected, and infection will also gradually increase in the farm during the breeding period, which may lead to the brood's infection with *Salmonella* (2). Other studies conducted on the risk factors of infection with *Salmonella* in poultry farms have also shown the older age of the brood to be one of the risk factors of *Salmonella* (20-24).

There was a significantly larger number of poultry houses in the infected farms than the uninfected ones in this study (P=0.037). In most cases, breeder farms adopt strict measures concerning the biosecurity principles and standards and it is highly important to take such preventive measures to prevent the entry of infectious agents including the Salmonella bacteria to poultry breeding farms (25, 26). Taking preventing measures against *Salmonella* infection such as changing clothes, taking shower, sterilizing boots and controlling rodents and insects would be more difficult at poultry farms with multiple poultry houses. Large breeder farms with multiple chicken houses, especially the ones with multi-age production systems are more likely to become infected with *Salmonella* at any points prior or after production. In other words, the greater the number of broiler poultry houses, the probability of isolating *Salmonella* bacteria from at least one house of the farm will be higher. Larger number of farms have also been previously shown to be associated with increased risk of Salmonellosis in poultry breeder farms (27, 28).

There are some inconsistancies between the results of our study and previous studies. For instance, the findings of this study indicate that there is no relationship between Salmonellosis and the capacity of chicken farms, whereas in other similar studies it has been shown to be a main factor for *Salmonella* infection in a poultry house (16-18, 20). Another risk factor was the different age system. We expected to see a statistical relationship with this factor and *Salmonella* infection, however according the final logistic model there was no significant association in this case (20, 21, 27). We think that it may be due to our small positive samples which decreased the power of our study to detect statistically significant difference between *Salmonella* infection and the different age system. The existence of proper fences around the farm and being equipped with manure collection equipment are two management factors which we expected to be associated with *Salmonella* infection, but the present research has reached contradictory

results regarding this relationship. It should be noticed that more than 90% of sampled farms had proper fencing and were equipped with manure collection equipment. So it was a consistent factor between farms, making it almost impossible to detect differences between positive and negative farms. The two factors, breed (28) and season (29, 30) have also been shown by few studies to be associated with *Salmonella* infections, whereas these two factors were shown by this study to be insignificant in Salmonellosis at broiler breeder farms .

In conclusion, due to the high prevalence of Salmonellosis at breeder farms in Iran and the limited impact of management factors on Salmonellosis in these farms, it is necessary to adopt effective measures to prevent and control *Salmonella* at broiler breeder farms in this country. More effective control measures, including vaccination of broods, can help reduce infection in poultry farms and consequently along the chicken meat production chain. Regular sampling for early detection of infected broods is extremely important in this regard. As the results obtained in this study show that there is a greater possibility of Salmonellosis with the greater age of broods, prioritizing sampling at the farms of older breeders with shorter intervals is recommended.

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